THE REGENERATION OF WHOLE POLYPS FROM ECTODERMAL FRAGMENTS OF SCYPHISTOMA LARVAE OF AURELIA AURITA [1]

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Gilchrist (1937) reported that isolated ectodermal fragments of scyphistoma larvae of *Aurelia aurita* reconstituted an entire organism. The report did not include a histological study and gave no indication of the cell type involved in the formation of the new endodermal layer. Gilchrist conjectured that interstitial cells were responsible for the formation of the new layer. In view of the uncertainty of the role of interstitial cells in hydroid regeneration and the fact that some features of reconstitution may occur in their absence (Brien *et al.*, 1953; Normandin, 1960), Gilchrist's experiments with scyphistomae of *Aurelia* were repeated and an attempt was made to trace the origin of the endoderm by means of a histological study of a staged series of reconstituting ectodermal isolates.

The observations here presented indicate that *Aurelia* scyphistomae have no cells which conform to the characteristics of typical hydroid interstitial cells. A count of mitoses reveals that somatic ectodermal cells are the most actively dividing cells and that they give rise to a population of cells (amoebocytic in appearance) which in turn become the new endodermal cells.

Materials and Methods

Scyphistoma larvae of the marine scyphozoan, *Aurelia aurita*, were obtained through the courtesy of Dr. Sears Crowell, and were grown in the laboratory at room temperature. They were kept in small fingerbowls or plastic Petri dishes containing filtered sea water obtained from Woods Hole, Mass. The larvae were fed each morning for 30 to 60 minutes on freshly-hatched brine shrimp (*Artemia*). The *Artemia* were hatched in sea water, rinsed several times, and introduced into the cultures of *Aurelia*. After the animals had fed, the culture water was replaced with freshly filtered sea water. Water was changed again each evening.

Animals were removed from the stock culture, placed in Syracuse dishes containing filtered sea water, and starved for 24 hours prior to operation.

All operations were performed with the aid of a dissecting microscope at a magnification of either 15 × or 20 ×. Peduncle, hypostome and tentacles were removed with sharpened surgical needles. The remaining cylinder of tissue was slit

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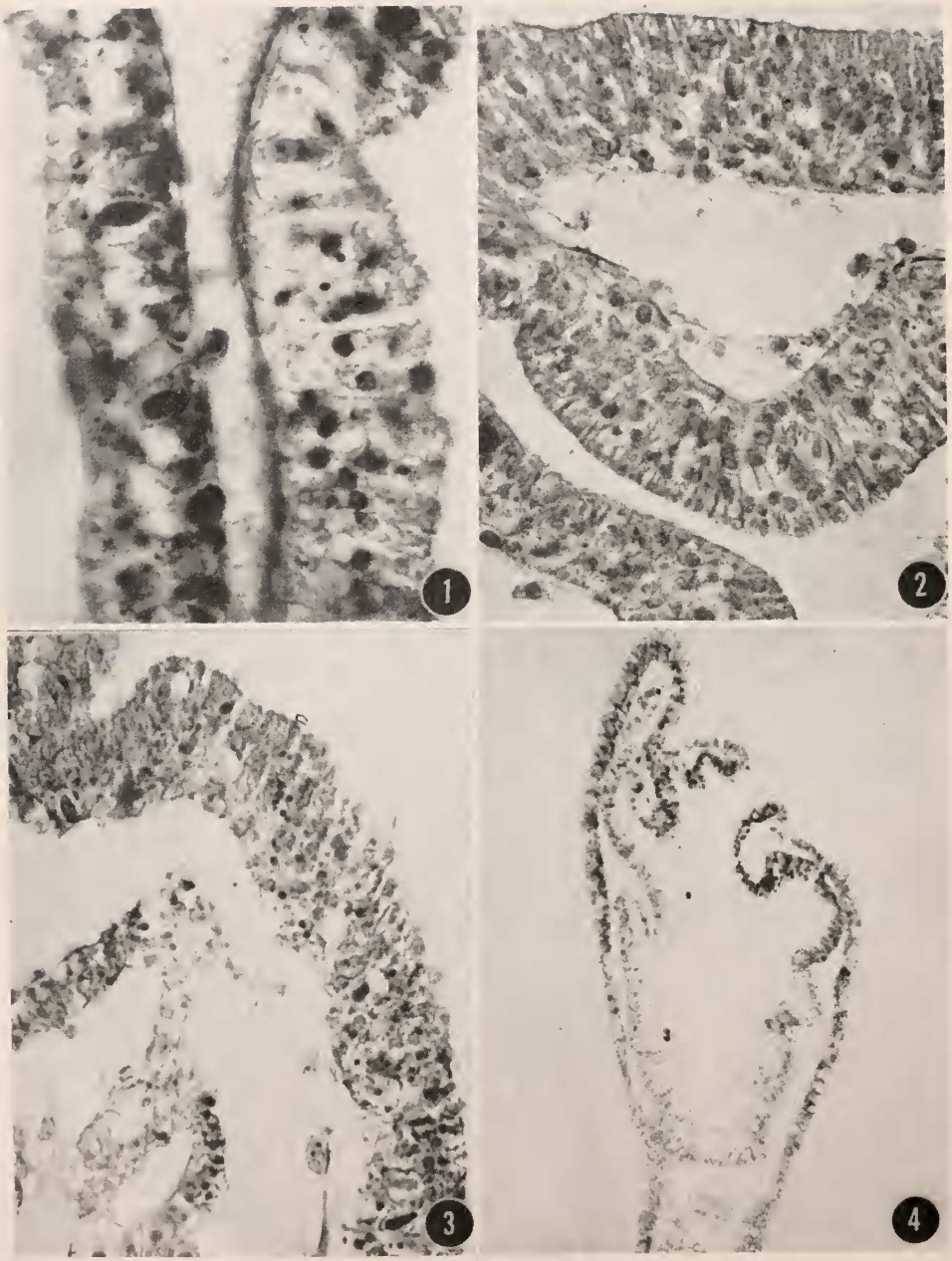

FIGURE 1. Cross-section through the mid-body region of *Aurelia*, showing ectoderm (on left), endoderm, mesoglea and an amoeboid cell. (× 840.)

FIGURE 2. Cross-section of an ectodermal fragment six hours after its separation from the endoderm, showing several amoeboid cells in the lumen. (× 520.)

FIGURE 3. Cross-section of a two-day-old regenerating ectodermal fragment. A distinct endoderm is present, and an amoeboid cell can be seen between the two layers. (× 520.)

lengthwise, flattened, and oriented so that the endodermal layer was facing upward. The tissue was then cut into six strips, each about 0.5 mm. wide, and the length of the original cylinder (about 1 mm.). The rectangular piece of tissue was held with a #5 watchmaker's forceps while a cut was made to the level of the mesoglea with a surgical needle. The endoderm was separated from the ectoderm by teasing and cutting through the mesoglea.

In *Aurelia,* the ectoderm is unpigmented, while the endoderm contains an orange pigment. This pigment is a useful diagnostic marker which helps to insure the elimination of all contaminating endoderm. The pigment marker, plus the thick mesoglea which separates the two layers, enables one to be certain of isolation of pure ectoderm. The endoderm-free isolates were transferred in a pipette to plastic Petri dishes containing filtered sea water. Fragments were sacrificed immediately after the operation (time 0), at 2, 4, 6, 8, 10, 12, 18, 20, 22, 24, 30, and 36 hours, and at 2, 3, 4, 5, and 6 days.

The specimens were fixed in one of the following fluids: Bouin's, Gilson's prepared with sea water, Carnoy's, Kleinenberg's, and Zenker's acetic. Bouin's and Gilson's were most useful, and were used in the majority of the work.

Animals were fixed for one-half to one hour, dehydrated in a graded ethanol series, cleared in cedarwood oil, and embedded in either 60–62° C. mp paraffin with 10% beeswax, or in 56–58° C. mp paraffin. Sections were cut at 7 microns.

Several stains were employed: toluidine blue (0.25% aqueous solution at pH 5), iron haematoxylin, Feulgen's, Azure B, Mallory's triple aceto-orcein. Only the first three successfully stained the sections of *Aurelia,* and they were used in most of the experiments.

Results

Preliminary data

Thirty-two isolated ectodermal fragments were cultured in filtered sea water. All of these fragments formed complete hydranths. Twelve started to capture freshly hatched *Artemia* on their sixth day, the other 20 on the seventh. Ingestion of the shrimp began one day later. In addition, 41 specimens, all having a longest dimension of at least 0.18 mm., were raised until they had formed at least one tentacle. Tentacles began to appear six or seven days after isolation of the ectoderm. Eleven additional fragments, all less than 0.18 mm. long, that did not show any signs of developing tentacles by day seven, were scored as negative and were discarded.

Normal histology of Aurelia

The intact scyphistoma of *Aurelia aurita* is a two-layered, sac-like animal. In the outer, single-celled layer of ectodermal cells there are various epithelial derivatives: epitheliomuscular cells, cnidoblasts, nerve cells, columnar epithelium. The inner endodermal layer is also one cell thick. Some endodermal cells are digestive, some glandular, and others muscular. The endoderm is invaginated into the

Figure 4. Longitudinal section through the mouth of a six-day-old regenerating specimen. The endodermal layer is complete, basophilic glandular cells surround the mouth, and a tentacle has formed. (× 235.)

lumen to form four longitudinal ridges, which enclose the gastric pouches. The two cell layers are separated by an acellular mesogleal layer (Fig. 1).

The base of the animal is further differentiated into a holdfast region in which the endodermal cells are enlarged and elongated. The apical region contains a mouth which is surrounded by a dense population of small, basophilic glandular cells in the endodermal layer. The mouth is ringed by a circle of tentacles, each of which bears several batteries of nematocysts.

Histology of regenerating ectodermal fragments

The development of an entire polyp from an ectodermal fragment from the mid-body region (see Fig. 1) was followed at intervals from 0 to 7 days.

(a) 0 hours: Fourteen specimens were fixed immediately after the ectoderm was separated from the endoderm. They consist of fragments of ectoderm, and some adhering mesoglea. The ectoderm appears as a single folded layer of columnar cells. Endoderm is not present in any of the sectioned specimens. An occasional large amoeboid cell is in the mesoglea of some specimens, but in no cases at a frequency of greater than 1 per 250 cells. Each explant contains a few thousand cells which are in a compact, though fragmented, mass.

(b) 2 hours: The three explants examined have lost their fragmented appearance, and have developed into a hollow mass of cells. The shape of the mass is irregular, but all of its contours are rounded. The inner margin of the ectoderm is lined with a thin layer of mesoglea.

(c) 4 hours: There is a slight increase in the number of amoeboid cells in the mesoglea of the three specimens examined. Some ectodermal cells appear to be necrotic. An occasional mitotic figure is visible in the outer margin of the columnar ectodermal layer.

(d) 6 hours: Fifteen specimens were fixed. Mitotic figures are more numerous in the ectoderm than at 4 hours, and there is an increase in the number of amoeboid cells. The latter cells are confined to the lumen, and are attached to the mesoglea. They exhibit long thin processes, are vacuolated, and have a large eccentric nucleus. No mitotic figures have been seen in them. The ectodermal cells are still all columnar, and arranged in an orderly fashion, similar to that observed in the normal animal (Fig. 2).

In some areas there is a necrotic mass containing many refractile inclusions and semi-lunar, Feulgen-positive bodies. The refractile bodies appear to be breakdown products of cnidoblasts. Mitotic activity is not found in the area surrounding the necrotic masses, and amoeboid cells are rare in this region.

(e) 8 hours (3 specimens): There are areas in which the lumen is obliterated by necrotic masses. In non-necrotic portions of the animal there are invaginations of ectoderm; the population of amoeboid cells is most dense in these regions of invagination.

(f) 10 hours (3 specimens): The lumen is large, and the areas of necrosis are less numerous. The number of amoeboid cells is about the same as at six hours.

(g) 12 hours (14 specimens): Mitotic activity is still confined to the ectoderm, and is occurring at a surprisingly high rate of about 1/250 cells. There is a marked increase in the amount of mesoglea.

(h) 18 hours (11 specimens) : The mitotic rate is more than double that seen at 12 hours. Mitotic activity is confined almost exclusively to the ectoderm; in all of the sections studied only one amoeboid cell was seen in the process of division.

(i) 20 hours (3 specimens) : No apparent change is visible.

(j) 22 hours (3 specimens) : Amoeboid cells are numerous, and are found scattered throughout the lumen. Several are packed densely near the point of invagination of the ectoderm. The ectoderm is now extremely regular, with the long axis of each cell pointing to the center of the enclosed area and the nuclei all basal. Cnidoblasts have appeared.

(k) 24 hours (18 specimens) : Some areas of the lumen are densely populated with amoeboid cells. The mitotic rate has not changed noticeably since 18 hours, and mitotic activity is still confined to the ectoderm.

(l) 30 hours (2 specimens) : The amoeboid cells are beginning to line up end to end, or to arrange themselves into small groups. They are confined to the lumen, and are visible on both sides of the mesogleal layer, with which they are in contact. Some cells have extended long filamentous processes toward neighboring cells, and are in contact with these neighbors via the resulting bridges. There is little mitotic activity. Necrotic areas are no longer visible.

(m) 36 hours (2 specimens) : The amoeboid cells are lined up in tandem, and form a distinct second layer, in which some typical endodermal cells may be seen for the first time.

(n) 2 days (11 specimens) : There are two distinct layers of cells; the outer is ectodermal, the inner is endodermal. Mitotic activity is confined to the ectoderm and is becoming more frequent (Fig. 3).

(o) 3 days (13 specimens) : Endodermal mitoses are becoming apparent, but are less frequent than ectodermal mitoses. An endodermal layer is distinct and, with the exception of one specimen, amoeboid cells are very rare. The exceptional specimen has one area with abundant amoeboid cells and no endodermal layer. Except for the one area this specimen has a distinct endodermal layer lacking amoeboid cells.

In the other specimens at this time the endoderm is a distinct, coherent layer, and, in usual fashion, is separated from the ectoderm by the mesoglea. A mouth is beginning to form at one end of the mass.

(p) 4 days (12 specimens) : There is little change from three days. Amoeboid cells are rare in all specimens.

(q) 5 days (6 specimens) : The animals are forming apical outpocketings (consisting of both ectoderm and endoderm) in the region of the hypostome. There are invaginations of the endoderm which resemble gastric pouches.

(r) 6 days (3 specimens) : The mouth has formed, and the area of the hypostome is surrounded by small, basophilic glandular cells. At the opposite end of the animal, the endodermal cells have enlarged into typical vacuolated stalk cells. Tentacles, complete with batteries of nematocysts, are present, although they vary in number and size from specimen to specimen. Mitotic activity is found in both ectoderm and endoderm (Fig. 4).

(s) 7–10 days: Some time during this period the regenerant begins to feed. Several specimens, including at least one from each set of experiments, have been followed for a few weeks, until they bud off a new scyphistoma.

Discussion

Gilchrist (1937) assumed, with no microscopic evidence to support his contention, that the newly formed endodermal cells were formed from interstitial cells in his ectodermal isolates of *Aurelia*. The present study has failed to reveal typical interstitial cells in either an intact scyphistoma, or during any stage of reconstitution. During regeneration, large amoeboid cells, similar to ones seen only infrequently in the intact animal, begin to appear. These cells do not have the typical basophilic character of a standard interstitial cell; instead, they are undistinguished cells, with no definite shape or characteristic cytoplasmic constitution.

These amoeboid cells could have come from either pre-existing amoeboid cells or from somatic ectoderm cells. The evidence indicates that the latter is the case. This evidence is based on fourteen ectodermal isolates which were selected at random during the experiments and fixed immediately after preparation. Nine of these contained no amoeboid cells; the other five had such cells but in no case was there more than one for every 250 ectoderm cells. Since all isolates of pure ectoderm which were larger than 0.18 mm. in length regenerated completely, we can assume (if the fourteen isolates are representative) that initial presence of amoeboid cells is not required for formation of an endodermal layer. Further, there was a consistent increase in the relative number of amoeboid cells with time after isolation, and such cells were found in all specimens fixed four hours or longer after preparation. Since this increase occurred in the absence of significant mitotic activity in the amoeboid cell population and was accompanied by a high mitotic rate of the ectoderm, we are led to the conclusion that the amoeboid cells, which eventually form the endodermal cells, are derived from the somatic ectoderm cells.

The source of cells in regeneration has not been clearly established. Attempts to link the new cells to interstitial cells or neoblasts often involve x-irradiation. Such treatment inhibits migration of interstitial cells, and regeneration ceases (Brien and Reniers-Decoen, 1955; Burnett, 1961; Puckett, 1936). One can conclude that interstitial cells are necessary for regeneration only if one assumes that x-irradiation does not affect somatic cells. Most studies of regeneration involve cases where all somatic cell types are represented. These are known to divide, and can be the source of the new cells. The question at issue is whether a somatic cell of one type is capable, regardless of its pathway, of forming a cell of another type. It has been proposed that, once a cell has received and used the information which determines its adult type, it loses its capacity to become another type of cell. The situation can best be assessed where one tissue type is eliminated. This has been done in the present study. *Aurelia* possesses no reserve cells, and in the present study it has been shown to be capable of reconstituting an endodermal layer when this layer is absent. The evidence is strong that somatic ectoderm can give rise to somatic endoderm, by first losing its characteristics and becoming an "indifferent" amoeboid cell.

Summary

1. Isolated ectodermal fragments of *Aurelia aurita* scyphistomae regenerate into complete hydranths. *Aurelia* possesses no interstitial cells, but large amoeboid cells appear during reconstitution.

2. The endoderm forms by differentiation of the amoeboid cells, which have probably arisen by dedifferentiation of the ectoderm.

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